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LITHIUM ION "CYCLOTRON RESONANCE" MAGNETIC FIELDS DECREASE SEIZURE ONSET TIMES IN LITHIUM-PILOCARPINE SEIZED RATS

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The cyclotron resonance equation predicts that the frequency of an applied magnetic field that might optimally interact with a single ion species may be computed as a function of the charge-to-mass ratio of the ion and the strength of the background static magnetic field. The present study was undertaken to discern the applicability of this equation for optimizing lithium ion utilization in the rat, as inferred by the predicted magnetic "ion resonance" field-induced shift of lithium's dose-dependent curve for seizure onset times (SOTs) when combined with the cholinergic agent pilocarpine. Groups of rats were administered 1.5 thru 3 mEq/kg lithium chloride (in 0.5 mEq/kg increments) and exposed to reference conditions or to one of three intensities (70 nanoTesla, 0.8 microTesla, or 25 microTesla) of a 85 Hz magnetic field calculated to resonate with lithium ions given the background static geomagnetic field of approximately 38,000 nanoTesla (0.38 Gauss). A statistically significant quadratic relationship for SOT as a function of magnetic field intensity (irrespective of lithium dose) was noted: this U-shaped function was characterized by equal SOTs for the reference and 25 microTesla

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groups, with a trend toward shorter SOTs for the 70 nanoTesla and 0.8 microTesla groups. Although not predicted by the equations, this report extends other findings suggestive of discrete intensity windows for which magnetic field frequencies derived from the cyclotron ion resonance equation may affect ion activity.

Keywords cyclotron resonance, epilepsy, magnetic fields, rats, seizures

Any system, mechanical or electromagnetic, that is characterized by a distinct periodicity (and thus frequency), will respond in a non-linear fashion when an external signal is applied that is tuned to the same frequency (Liboff et al., 1987). Liboff (1985) proposed that ion cyclotron resonance might underlie the biological and cellular effects of extremely low frequency and low intensity magnetic fields in accordance with this principle of non-linear amplification. By equating the Lorentz force imposed on a particle in a static magnetic field with the particle's centripetal force as it moves through a constrained space (e.g., a membrane channel) the gyrofrequency of the ion (the rate at which it completes its circuitous path through the channel (f)) is a function of the charge-to-mass ratio of the ion (q/m) and the intensity of the static magnetic field (B). Succinctly this relationship is expressed as $f = (l/27\pi)(q/m)(B)$.

The model predicts that an applied magnetic field of weak intensity but tuned to the gyrofrequency of the biologically active ion will deliver energy at a rate that is maximally transferred to the ion, thereby overcoming the thermal noise (kT) constraints considered problematic for low energy magnetic field transduction. Transferring energy to the biologically important ions is thought to increase the probability of their transport and/or the velocity of their transport (ion pumping efficiency) across membranes. Because each ion has a unique charge-to-mass ratio, magnetic fields may be tuned to specifically interact with one ion species at a time.

Previous work by Thomas et al. (l987) suggested the role of lithium ion resonance in an operant task using 30 min presentations of a 55 Hz sinuisoidal field after bucking the Earth's static field to 0.27 Gauss (thereby satisfying the requirements of the resonance equation). However, this study did not directly measure lithium activity. Subsequent experimental work by McLeod et al. (1987) demonstrated that the marine diatom *Amphora coffeaeformis,* whose

calcium-dependent motility is strongly governed by a critical threshold of calcium in the culture medium, became motile in a calcium poor medium when a magnetic field tuned for calcium resonance was applied to the cultures. The enhanced motility may have been related to increased efficiency of membrane calcium transporters that maximized the use of the calcium in the calcium poor medium, or alternatively may have been related to an increased mobilization of internal calcium stores.

Honchar et al. (1983) demonstrated that an injection of 3 mEq/kg lithium chloride (LiCl) 24 h prior to pilocarpine (a muscarinic agonist) reduced the dose of pilocarpine required to cause the overt display of seizures in rats from 380 mg/kg (Turski et al., 1983) to 30 mg/kg. Persinger et al. (1988) demonstrated that the LiCl injection need only precede the pilocarpine injection by 4 h and established the relationship that ascending doses of lithium increased both the probability and the severity of the overt convulsion evoked by pilocarpine. If the equations for ion cyclotron resonance, which have been utilized to calculate calcium frequency fields that effect increases in calcium activity in cultured cells, may be generalized to lithium ions, as suggested by Thomas et al. (1987), then application of a magnetic field tuned for lithium ion resonance subsequent to LiCl injection should facilitate pilocarpine-induced seizures as inferred by decreases in seizure onset times. The magnetic field tuned for lithium resonance was applied at several intensities as cumulative evidence suggests that the biological effects of magnetic fields with specific frequencies only occur at specific intensities (Bawin & Adey, 1977; Bawin et al., 1978, Liboff et al., 1987; Prato et al., 1995).

METHOD

Subjects

Male Wistar strain rats (n = 59) ages 100–120 d and mean mass of approximately 500 grams were entered into the study. Prior to the experiment all rats were maintained with food and water *ad libitum* while housed 3/cage in standard colony metal cages. The L:D cycle

was 12:12 with photophase onset at 0730 h local time, The experiment was conducted during the mid to late photophase.

Lithium Ion Resonance

The charge-to-mass ratio of the monovalent lithium ion (unhydrated) is 0.14 (in units of e/u). The magnitude of the static geomagnetic field within the exposure coils employed in these studies was approximately 38,000 nT (0.38 Gauss) (FM-300 Fluxgate Magnetometer). Using the cyclotron resonance equation presented earlier, the frequency for lithium ion resonance is approximately 85 Hz.

Magnetic Field Apparatus

The experimental setting consisted of four plastic cages, each 32 cm × 36 cm × 45 cm. Specific details of this equipment have been documented elsewhere (Persinger et al., 2000). Two of the cages were inserted into wooden frames wrapped around their long axes with 24-gauge (AWG) wire (30 ohm). These coils were separated by 5 cm. The third plastic cage was placed 50 cm underneath the two coils and the fourth cage was placed on the opposite side of the room. The first coil was connected to a Model EU 81A Heath-Schlumberger function generator set to deliver an 85 Hz sinusoidal field. The intensity of the field at the level of the rats in the active coil, as determined by a Metex Multimeter with a magnetic probe was 25 microTesla. The second coil was not connected to the equipment but because of proximity to the active coil received a 0.8 microTesla induced field. The AC magnetic field intensity in the third cage (underneath the two coils) was 70 nanoTesla, and the magnetic field intensity in the fourth cage (the reference condition) was below measurable levels (<10 nanoTesla).

Lithium/Pilocarpine Injections and Field Exposure Procedure

Rats were taken from their home cages and given subcutaneous (s.c.) injections of 1.5 (n = 17), 2 (n = 20), 2.5 (n = 12), or 3 mEq/kg (n = 10) LiCl (1 cc/kg). The rats were then placed 3/cage (no

more than one rat of each lithium dose per cage per session) in one of the four experimental cages and received 4 h presentations of the 85 Hz lithium-modeled sinusoidal magnetic fields (70 nT: $n = 14$; 0.8 uT: $n = 14$; 25 uT: $n = 15$) or 4 h exposures to the reference condition ($n = 12$). After the allotted time rats were quickly relocated to the room in which seizures were induced and given s.c. injections of 30 mg/kg pilocarpine (1 cc/kg). The time of seizure onset (to the nearest second), as defined by the display of at least a Racine level V seizure (defined by loss of balance and falling, which invariably follows the display of clonic forelimb convulsions and often precedes generalized clonic convulsions) (Racine, 1972) was recorded manually. Immediately after seizure onset rats were administered acepromazine (25 mg/kg; s.c.) to facilitate survival (Harrigan et al., 1994). These rats were then entered into experimental protocols for other researchers.

Statistical Analyses

Analysis of variance (ANOVA) was the primary statistical tool. *Post hoc* analyses included Student Neuman-Keuls (SNK), with criterion for significance set at $p < .05$. To examine dose-dependence for SOTs as a function of lithium concentration or magnetic field intensity polynomial (trend) analyses were completed. All analyses were completed with SPSS software on a VAX 4000 computer.

RESULTS

Chi-square analyses indicated there were no statistically significant deviations from chance expectancy for occurrence of overt seizure (0 = no overt seizure; 1 = overt seizure) for rats treated with one of four doses of lithium [$\chi^2(3) = 2.75$, ns] or given applications of one of four intensities of the 85 Hz magnetic fields [$\chi^2(3) = 4.14$, ns].

Two-way analysis of variance for seizure onset time as a function of one of four lithium doses and one of four magnetic field treatments revealed statistically significant differences between lithium-treated groups [$F(3,39) = 4.52$, $p < .01$, multiple $R = 0.38$] and between magnetic field-treated groups [$F(3,39) = 5.31$, $p < .01$, multiple

$R = 0.41$]. Polynomial analyses indicated that as the dose of lithium increased there was a linear decrease [$F(1,51) = 5.71$, $p < .05$] in seizure onset times (data not shown). *Post hoc* analyses (SNK, $p < .05$) indicated a statistically significant difference between SOTs for rats exposed to the reference condition, and rats exposed to the 70 nanoTesla and 0.8 microTesla, 85 Hz magnetic fields; these latter groups did not differ significantly from one another (Figure 1). The quadratic term of the *post hoc* was statistically significant [$F(1,51) = 9.80$, $p < .01$] and indicated a U-shaped function wherein rats exposed to reference conditions or the 25 microTesla, 85 Hz field, displayed a trend toward relatively longer SOTs than rats exposed to the 70 nanoTesla or 0.8 microTesla, 85 Hz magnetic field.

DISCUSSION

Given the charge-to-mass ratio of the lithium ion, and the 0.38 Gauss DC (static) geomagnetic field inside the coils at Sudbury latitude, these parameters were entered into the ion cyclotron resonance equation (Liboff, 1985) and the authors calculated that a 85 Hz sinusoidal

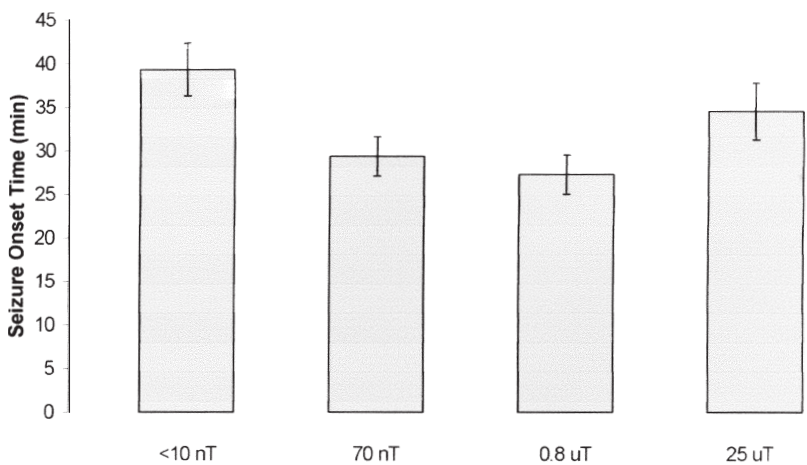

FIGURE 1. Mean seizure onset times for rats given the epileptogenic compound pilocarpine following 4 h exposures to reference conditions or one of three intensities of 85 Hz magnetic fields. Each group contained the same proportions of rats given each of the four doses of lithium. Error bars indicate standard errors of the means.

magnetic field might optimally affect lithium ion utilization, as measured by changes in seizure onset times in lithium-pilocarpine treated rats. This frequency affected SOT in the rat, but only for specific intensities of the applied AC field.

A U-shaped function for SOT as a function of magnetic field intensity was noted and was best described as a trend toward lower SOTs for 70 nanoTesla and 0.8 microTesla magnetic field treated groups relative to reference groups and those rats exposed to the 25 microTesla condition. However, in the absence of applied 85 Hz synthetic magnetic field treatments, the typical SOT for 3 mEq/kg lithium treatment followed 4 or 24 h later by pilocarpine treatment (SOTs for these intervals do not differ significantly from one another) is 23.0 min (SD = 6.4 mm; n = 96) (McKay, unpublished observations). In the present study several low (<3 mEq/kg) levels of lithium treatment were employed that ostensibly inflated group SOTs. However, each magnetic field treatment group contained equivalent numbers of rats treated with low-doses of lithium and thus SOTs were likely homogeneously inflated for all groups, thereby justifying the discrepancy between the reference group SOTs in the present study and unpublished SOT data.

Cumulative evidence indicates that the effects of magnetic fields on biological systems are dependent on the waveform (shape) of the applied magnetic field. Investigations into the pharmacological mechanisms mediating the effects of low-intensity and extremely low frequency (ELF) magnetic fields have discerned the role of modulations in opioid activity following magnetic field applications. Complex ELF magnetic fields, defined by their non-sinusoidal or physiologically relevant waveforms, have been shown to effect increases in delta-opioidergic activity (Thomas et al., 1998), whereas 60 Hz powerline (sinusoidal) magnetic fields have been shown to attenuate opioid activity (Tysdale et al., 1991). Because the effects of magnetic fields are specific to the shape of the waveform it is difficult to generalize findings from one study to another wherein waveforms with different shapes were employed.

It may be relevant that systemic administration of opioid agonists, including morphine, enhanced the seizure-evoking properties of pilocarpine, whereas systemic administration of opioid antagonists, including naloxone, attenuated the effects of pilocarpine (Turski

et al., 1985). Turski et al. (1986) reported that intranigral infusions of morphine markedly reduced the required dose of pilocarpine to evoke limbic seizures. In fact, limbic epileptic seizures that generalize through the brainstem, particularly the substantia nigra, are phenotypically characterized by wild running and jumping due to alternating tonic/clonic activity of extensor muscles (Stewart et al., 2001). Although this phenotype is readily observed when pilocarpine is administered during the mid-scotophase, it is rarely observed during the late photophase when the animals of this study were injected. Over the course of this study it was noted a disproportionate number of field-exposed rats displayed this unusual seizure phenotype, an observation that may be relevant in the context of a magnetic field-induced opioid-augmented process.

Kavaliers et al. (1998) have reported that 60 Hz magnetic field effects resemble systemic administrations of pharmacological agents that effect increases in nitric oxide activity. Interestingly, acute administrations of the neuronal nitric oxide synthase (nNOS) inhibitor 7-nitro indazole have been shown to attenuate the severity of pilocarpine-induced seizures (Van Leeuwen et al., 1995), whereas a similar nNOS inhibitor has been shown to increase the latencies to seizure onset (Dzoljic et al., 1997). Opposite effects have been reported for the nNOS inhibitor L-NAME (Starr & Starr, 1993; Maggio et al., 1995; Del-Bel et al., 1997). Consistent with the complex regulation of pilocarpine-evoked seizures by specific doses and structures of systemically administered compounds, it may be that ELF sinusoidal magnetic fields, with known nitric oxide-like effects (Kavaliers et al., 1998), only influenced SOTs at specific intensities (electromagnetic doses). Discerning the role of waveform structure for SOTs was beyond the scope of the present study.

The ion cyclotron resonance equation proposed by Liboff does not provide a means to calculate the amplitude (intensity) of the applied sinusoidal field. At the equation-predicted cyclotron resonance frequency for calcium ions, Liboff et al. (1987) have reported that incorporation of radiolabeled calcium into human lymphocytes occurs maximally at approximately 21 microTesla; the window of efficacy around 21 microTesla was approximately ± 10 microTesla and asymptoted to zero incorporation very quickly outside these parameters. The authors postulated that discrete intensity windows

likely existed for specific frequencies, as the optimal intensity would activate in a facilitative manner a particular compartmentalized calcium store thereby increasing calcium uptake as noted when assayed, and other intensities would activate in an inhibitory manner other compartmentalized stores of calcium ions, thereby decreasing (or inhibiting any change of) calcium ion uptake.

Bawin and Adey (1977) and Bawin et al. (1978) have reported that 450 MHz fields amplitude modulated at 16 Hz increased radiolabelled calcium efflux only at specific intensities of the applied fields. The suggestion was put forth that frequency and intensity windows were related to a filtering mechanism intrinsic to the biological system. It is uncertain from the results of the present study whether such a filtering mechanism exists at the level of the lithium ion, or whether the filtering mechanism is intrinsic to a larger pharmacological pathway implicated in the transduction of lithium's cellular effects.

This intensity dependence reported by Liboff et al. (1987) for specific frequencies of the AC field is consistent with the resonance-like magnetic field inhibition of opioid-induced thermal analgesic responses reported by Prato et al. (1995). While holding DC intensity constant, and the frequency of the applied AC magnetic field constant, specific but non-successive intensities of the applied AC field attenuated opioid-evoked analgesia, and while holding DC intensity constant, and holding AC intensity constant, specific but non-successive frequencies of the applied AC field were effective in attenuating opioid-evoked analgesia (Prato et al., 1995)

After transforming their data to fit a Bessel function, Prato et al. (1995) suggested that their data indicated that the ratio of the intensity of the applied magnetic field relative to the static (DC) magnetic field on which the AC field was superimposed was important, as linear relationships between applied intensity and magnitude of effect (which would be consistent with a current induction mechanism) were not evident. This type of resonance mechanism, which has not contemporaneously been definitively articulated, may have underlain the intensity-dependent findings for SOT in the present study. In the context of the work by Prato et al. (1995), it is clear that such a resonance-like effect is not mutually exclusive from magnetic field-evoked modulations in opioid or nitric oxide activity,

as the resonance-like coupling to biological systems itself evokes changes in these pharmacological pathways.

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